J. Mar. Biol. Ass. U.K. (2000), **80**, 785–788
Printed in the United Kingdom

Divisional morphogenesis in the marine ciliate *Holosticha warreni* (Ciliophora: Hypotrichida)

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The cortical development of the marine hypotrichous ciliate *Holosticha warreni*, found in coastal waters near Qingdao, China, was investigated using the protargol impregnation method. In the proter, disorganization of the parental adoral zone of membranelles and undulating membranes contributes to the formation of its oral primordia which replace the parental buccal apparatus completely. Cirral anlagen in both division parts derive from the breaking of primary primordia. Most midventral cirri join in the formation of these primordia which occurs *de novo* separately from the oral primordia. Each of the 11 to 13 oblique streaks divides into three segments (new cirri) while the last two anlagen produce four each. Two frontoterminal cirri derive from the posteriormost anlage. The marginal rows develop from the parental structure. The generation mode of dorsal kineties is of the 'one group type' without forming caudal cirri.

INTRODUCTION

Patterns of morphogenesis may provide valuable information about interrelationships among ciliates, especially at higher taxonomic levels (Corliss, 1968). More recently, morphogenetic data have been used to discriminate between morphologically similar hypotrich ciliates (see Foissner, 1996).

Holosticha is a well-known, species-rich hypotrich genus, commonly found in marine, limnetic and terrestrial biotopes, studies on which have concentrated mainly on its morphology, ecology, taxonomy and systematic classification (Kahl, 1932; Borror & Wicklow, 1983; Foissner & Didier, 1981; Foissner, 1982, 1984; Dragesco & Dragesco-Kernéis, 1986; Foissner et al., 1991; Song & Wilbert, 1997; Hu & Song, 1999). Morphogenetic data, however, are available for only a few species (Hill, 1980; Martin et al., 1981; Hemberger, 1982; Wilbert, 1986; Foissner, 1996).

In the present paper, we describe the morphogenetic process in *H. warreni*, a recently described marine species (Song & Wilbert, 1997) collected from coastal waters near Qingdao, China and compare its morphogenesis with that of some of its congeners. The systematic implications for the genus *Holosticha* are briefly discussed.

MATERIALS AND METHODS

The organism and culture method were described in detail in Song & Wilbert (1997). The protargol staining method according to Wilbert (1975) was used to reveal the infraciliature of well-fed cells growing in raw cultures.

Drawings were performed under ×1250 magnification with the help of a camera lucida. To show clearly the changes during the morphogenetic processes, old cirri are depicted only by contour, whereas the new ones are blocked in. The terminology is mainly according to Borror & Wicklow (1983) and Corliss (1979).

RESULTS

Stages of divisional morphogenesis in *Holosticha warreni* are shown in Figures 1–3. The nuclear apparatus develops in the usual way for most hypotrichs (Figures 1F,H; 2B,D,F,H; 3I, arrows & L) and thus no further description will be given.

Stomatogenesis commences with the formation of anarchic groups of basal bodies close to the anterior transverse cirri, which develop subsequently to form the oral primordium (OP) of the opisthe (Figures 1A,C; 3C,D). Transverse cirri and the adjacent ventral cirri appear intact. At about the same time, part of the midventral cirri begins to disorganize to form the frontoventral-transverse (FVT) cirral anlagen.

By proliferation of basal bodies, the primordial fields enlarge and the old undulating membranes and posterior end of the parental adoral zone of membranelles (AZM) begin to disorganize to form new OP for the proter. About 11 oblique streaks (primary primordia) are formed as a ladder-like structure (Figure 1D, double-arrowheads). Most of the midventral cirri are gradually resorbed (Figures 1D & 3E).

Later, the anterior end of the oral primordium in both dividers bifurcates and the adoral membranelles are differentiated in the left fork, while the right one will become the anlagen of the undulating membrane (Figure 1E, arrows). Within the cirral anlagen, each streak seems to break into two, and thus two groups of cirral anlagen are formed (Figures 1E & 3F, arrowheads). During this stage, the anlagen for the marginal rows on the ventral side and three dorsal anlagen for both daughter cells appear within the parental structure. The old cirri are clearly involved in the formation of the anlagen (Figure 1E, double-arrowheads; F, arrows).

In the next stage (Figures 1G,H & 3G) development of the new adoral membranelles within the oral primordium

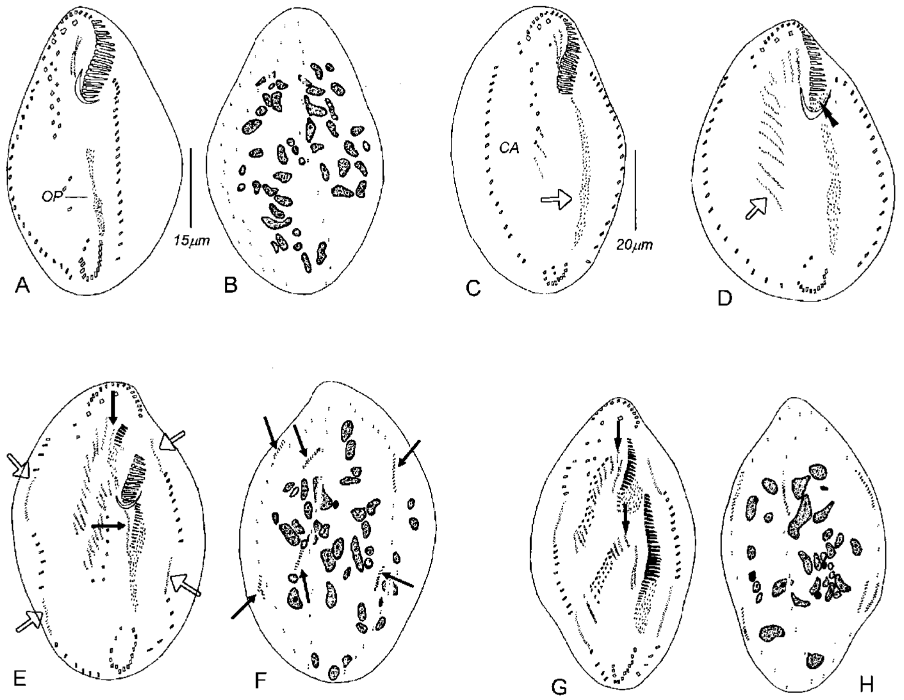

Figure 1. Early and middle stages of morphogenesis in *Holosticha warreni*. (A&B) Ventral and dorsal views of the same individual. (C) Ventral view, arrow indicating the oral primordium. (D) Ventral view, double-arrowheads marking the disorganization of posterior end of parental adoral zone of membranelles (AZM), while the white arrow indicates the cirral anlagen. (E&F) Ventral and dorsal views of the same cell, arrows marking anlagen of undulating membranes (UM-anlagen), white arrows indicating the marginal anlagen. (G&H) Ventral and dorsal views of the same individual, arrows marking the buccal cirrus splitting from UM-anlagen. CA, cirral anlagen; OP, oral primordium.

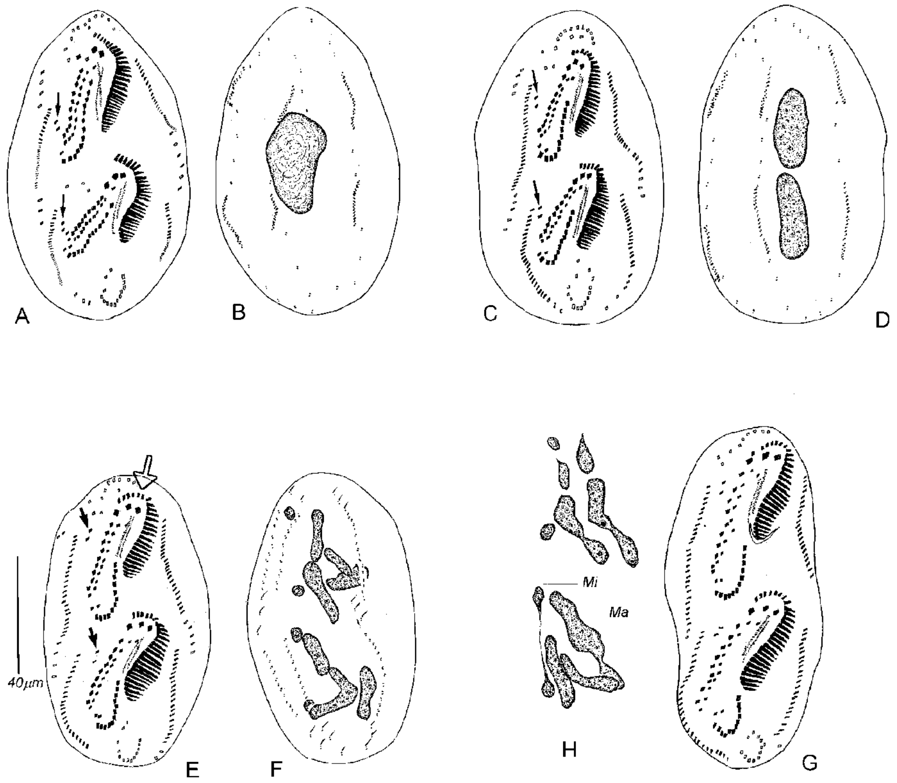

Figure 2. Late stages of morphogenesis in *Holosticha warreni*. (A&B) Ventral and dorsal views of the same individual; arrows indicating the frontoterminal cirri which are just migrating from the other cirral arrays. (C&D) Ventral and dorsal views of the same cell, note that the macronucleus is already in two parts; arrows marking the frontoterminal cirri. (E&F) Ventral and dorsal views, note that the parental adoral zone of membranelles (AZM) has been completely replaced by the newly-built one (white arrow in E); arrows in E indicating the frontoterminal cirri. (G) Ventral view to show the later stage which is just before cell division. (H) The same stage as in G, to show the macro- (Ma) and micronuclei (Mi); Ma are in active but irregular division.

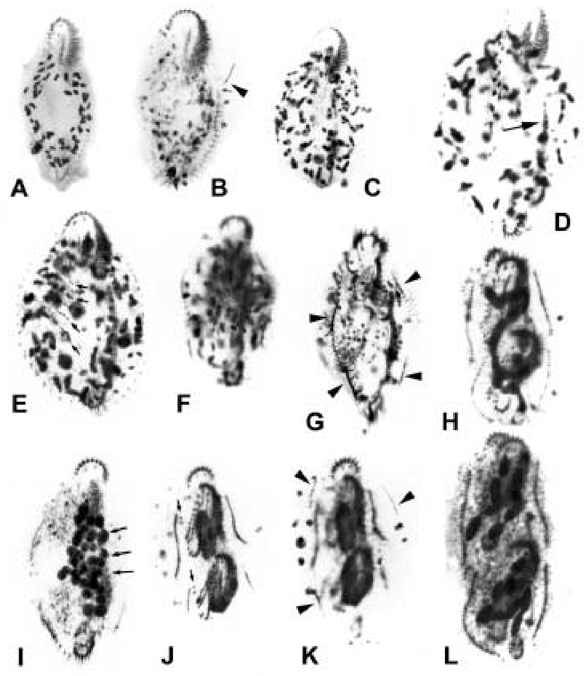

Figure 3. Photomicrographs of morphology and morphogenesis in *Holosticha warreni* (after protargol impregnation). (A) Ventral side, note that macronuclear segments are arranged peripherally. (B) To show the ejected extrusome (arrowhead). (C,E,F) Ventral side, showing primary cirral anlagen (arrowheads). (D) Ventral view, arrow marks oral primordium for the opisthe. (G) Arrowheads indicating the anlagen of right and left marginal row of proter and opisthe. (H) Ventral side, showing newly-formed cirri and adoral zone of membranelles in both daughter cells. (I) Note that the macronuclear segments begin to fuse. (J) Ventral side, showing the newly-formed cirri, arrows indicating the frontoterminal cirri. (K) Dorsal side, arrowheads showing dorsal kineties. (L) To show the macro- and micronucleus.

in both proter and opisthe proceeds posteriorly. A short anlage derives from the anterior end of the undulating membrane anlagen (UMA, arrows). Each of the cirral streaks begins to segment.

By now, the development of new AZM is almost complete in both daughter cells, and the anterior portion of the adoral membranelles begins to curve to the right (Figure 2A &3H). From the UMA one cirrus (leftmost frontal one) is generated, while the remaining part of the anlage split in both dividing cells, giving rising to the paroral and endoral membrane. Each of the cirral streaks divides into three segments (cirri), the two posteriormost anlagen producing four cirri each (Figure 2A): from the 'last' of these anlagen, two cirri begin to move anteriorly (Figure 2A&C, arrows).

Anlagen for marginal cirri and dorsal kineties stretch in both directions to replace the old structure (Figure 1A,B).

Finally, the migration of the newly-formed cirri commences. The two anteriormost cirri from the last cirral streak move in an anterior direction. The last segment of each streak contributes to the formation of the transverse cirri, which migrate posteriorly. Most of the remaining cirri are then arranged in a 'zig-zag' pattern with the exception of the frontal and buccal cirri (Figures 2C,E & 3J).

On completion of cell division, all cirri and the buccal apparatus are arranged in the species-specific pattern. Those parental cirri which do not participate in the formation of primordia will soon be resorbed. On the dorsal side, the newly built dorsal kineties have replaced the parental structure (Figures 1F,H; 2F & 3K, arrowheads).

The remaining morphogenetic events are very similar to those known for its congeners: the daughter-cells separate and, with the completion of the cytostome, become trophic cells.

DISCUSSION AND CONCLUSIONS

It has long been assumed that *Holosticha* is a monophyletic group because its members share very similar patterns of infraciliature. However, as a result of morphogenetic studies, we have reason to question this assumption (Martin et al., 1981; Hemberger, 1982; Foissner, 1996).

Morphogenetic data for this genus are known for only seven species (Foissner, 1996). The most detailed accounts are those for *H. multistilata*, *H. similis*, *H. diademata* (Hemberger, 1982), *H. musculus* (Martin et al., 1981), *H. geleii* (Wilbert, 1986) and *H. foissneri* (W.S., unpublished). Morphogenesis in *Holosticha warreni* exhibits significant differences when compared to that of these taxa, exhibiting a combination of the following characteristics: (1) in the proter, the adoral zone of membranelles is generated from a newly-built oral primordium, similar to that in *H. multistilata*, a relatively unusal character shared by several different groups of hypotrichs, e.g. *Keronopsis, Thigmokeronopsis*, *Metaurostylopsis* (Borror, 1972, 1979; Wicklow, 1981; Hemberger, 1982; Wirnsberger et al., 1985; Eigner, 1995; Petz, 1995; Foissner, 1996; Berger & Foissner, 1997; Song & Hu, 1999). This is in contrast with some of its other congeners, e.g. *H. diademata* and *H. geleii,* where the parental structure is completely or partly inherited by the proter, which hence resembles that in other urostylids, e.g. *Paragastrostyla, Uroleptus, Urostyla, Pseudourostyla* and *Periholosticha* (Borror, 1972, 1979; Jerka-Dziadosz, 1972; Martin et al., 1981; Hemberger, 1982; Wilbert, 1986; Wirnsberger, 1987; Wirnsberger et al., 1985; Song, 1990; Eigner, 1994, 1995; Foissner, 1996). (2) Unlike its congeners, the frontoventral-transverse cirral anlagen (FVT-anlagen) in the proter and opisthe generate from the breaking of the primary primordia. (3) All the frontoventral-transverse cirral anlagen contribute one transverse cirrus each, which make up the long row of the transverse cirri (vs only some posterior FVT-anlagen generate transverse cirri and thus usually forming a short cirral row).

From the results of the present investigation, and that of previous studies of morphogenesis in *Holosticha* (Martin et al., 1981; Hemberger, 1982; W.S., unpublished observations), a complex variety of morphogenetic patterns is revealed. These include at least three different patterns of AZM formation, two different patterns of FVT-anlagen formation, and two for the formation of the cirral anlagen. Although the evolutionary significance of these dissimilarities remains unclear (i.e. whether they are apomorphic or plesiomorphic characters), this level of diversity suggests that the genus *Holosticha* could be polyphyletic and, ultimately, may need to be subdivided into two or more genera. Morphogenetic data for a wider range of species within the genus are required in order to test this hypothesis.

This work was supported by 'The Natural Science Foundation of China' (Project no. 39770093) and 'exquota' travel grants from the Royal Society to W.S. and A.W.

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Submitted 18 February 2000. Accepted 18 May 2000.